Letter to the Editor

Delayed mammalian meat-induced anaphylaxis due to galactose-α-1,3-galactose in 5 European patients

To the Editor:

In 2008, severe anaphylactic reactions after first infusion of cetuximab were reported for the first time.[1] These episodes went against the idea that an allergic reaction needs previous contact with the allergen to develop sensitization. The authors demonstrated that preexisting specific IgE (sIgE) antibodies to galactose-α-1,3-galactose (α-gal) were responsible for the reactions.[1] The α-gal epitope is a well-known antigen widely expressed on tissues of nonprimate mammals and capable of inducing specific IgG and IgM antibodies in those species that do not express it, humans among them.[2] After the identification of α-gal-sIgE, a few cases of anaphylaxis, angioedema, and urticaria related to this oligosaccharide moiety have been described.[3-5] The clinical syndrome corresponds to a delayed hypersensitivity reaction that occurs 3 to 6 hours after the consumption of mammalian red meat. Additional features in these patients are the presence of sIgE to unrelated allergens such as cats,[6] dogs, and parasites.[7] Moreover, a characteristic geographical distribution within the United States was demonstrated.[1,8,9] Intriguingly, tick bites seem to underlie many cases of α-gal sensitization and the subsequent geographical differences in its prevalence.[9] To the best of our knowledge, only 2 patients with possible α-gal sensitization have been described in Europe until now.[4] In these cases, however, the presence of α-gal-sIgE was not demonstrated and the diagnosis was based on intradermal tests with a beef extract and/or cetuximab.[4]

Here we present 5 cases of delayed anaphylaxis to red meat in Europe (northwestern Spain). In these cases, α-gal-sIgE was demonstrated. Skin prick tests (SPTs) with a beef extract were positive, and inhibition experiments showed that beef-sIgE positivity was due to α-gal sensitization. Most patients reported a history of tick bites.

The 5 patients (4 males; Table I) were referred after several episodes of supposedly idiopathic anaphylaxis to the Allergy Clinics of Hospital Lucus Augusti (Lugo, Spain) (patients 1-4) and Hospital Clínico Universitario of Santiago (Santiago de Compostela, Spain) (patient 5) from August 2010 to January 2011. All patients reported repeated (>3) episodes of anaphylaxis several hours after eating beef and pork meat. All but 1 patient (patient 2) confirmed that they had been bitten by ticks in the previous months. One patient spontaneously suggested that his problems with meat had begun 2 weeks after having been bitten by a tick. SPTs to a panel of commercially available common inhalant and food allergens (*Dermatophagoides pteronyssinus*, *Phleum pratense* pollen, milk, egg, peanut, prune, shellfish, fish, *Anisakis simplex*, and beef meat) (Bial-Arístegui Laboratory, Bilbao, Spain) were performed in all cases. For the beef extract, raw beef was blended with PBS at pH 7.5 and extracted by magnetic stirring in agitation for 2 hours at 4°C. The sample was centrifuged at 7000*g* and then dialyzed against distilled water. The dialyzed extract was filtered by using a 7-kDa pore-size cellulose membrane and was lyophilized and dissolved in glycerinated buffered saline solution at 20 mg/mL. In addition, an SPT to cetuximab (Erbitux; Merck SL, Madrid, Spain) (5 mg/mL) was performed. sIgE to commercial allergens (beef, pork, lamb, rabbit,

TABLE I. Patient characteristics

	Patient no.				
	1	2	3	4	5
Sex	Male	Female	Male	Male	Male
Age (y)	44	86	41	26	62
Clinical syndrome	Delayed anaphylaxis	Delayed anaphylaxis	Delayed anaphylaxis	Delayed anaphylaxis	Delayed anaphylaxis
Onset of symptoms (h)	>3	4-6	3-5	3	4-5
History of tick bites	Yes	No	Yes	Yes	Yes
SPT to beef meat (mm)	12 × 10	5 × 3	4 × 4	10 × 6	8 × 5
SPT to cetuximab (mm)	9 × 6	14 × 10	7 × 5	8 × 4	9 × 6
Total IgE (IU/mL)	607	248	2080	291	98
Specific IgE (KU_A/L)					
Beef	20.6	9.7	18.3	2.92	1.23
Pork	8.68	6.47	17.7	1.81	1.24
Lamb	4.64	6.19	5.15	0.78	0.98
Rabbit	2.12	3.66	2.23	<0.35	0.72
Chicken	<0.35	<0.35	<0.35	<0.35	<0.35
Turkey	<0.35	<0.35	<0.35	<0.35	ND
Bovine seroalbumin	0.57	<0.35	<0.35	0.69	<0.35
Pork seroalbumin	<0.35	<0.35	<0.35	<0.35	<0.35
Cat dander	1.32	0.74	0.76	<0.35	0.65
rFel d1	<0.35	<0.35	<0.35	<0.35	<0.35
MUXF3	<0.35	<0.35	17.2	<0.35	<0.35
Ascaris	2.41	ND	2.26	<0.35	0.86
Echinococcus	1.35	2.78	6.96	<0.35	0.45
Anisakis	<0.35	<0.35	1.86	ND	<0.35
α-Gal	>100	18.1	43.6	23.1	9.7

MUXF3, The N-glycan from bromelain; *ND*, not performed; *r*, recombinant.

chicken, turkey, bovine seroalbumin, pork seroalbumin, cat dander, rFel d1, MUXF, *Ascaris lumbricoides*, *Echinococcus* spp, and *Anisakis simplex*) was conducted by using the ImmunoCAP-250 analyzer (Phadia, Uppsala, Sweden). The streptavidin CAP (Pharmacia Diagnostics, Uppsala, Sweden) technique was used to measure IgE antibodies to α-gal, as previously described.[1] Briefly, 50 μg of biotinylated α-gal (Glycotech Corporation, Gaithersburg, Md) was added to each CAP for 60 minutes at 37°C before adding undiluted serum. Results were measured in the ImmunoCAP-100 analyzer (Phadia).

The results of immunological tests in the 5 patients are displayed in Table I. SPTs were positive against cetuximab and the beef extract in all patients. No other positive response was achieved except for *Dermatophagoides pteronyssinus* in 1 patient with previous allergic rhinitis. The beef extract employed was routinely tested among more than 500 patients studied for chronic urticaria during 2010 with no positive results, thus making false-positive results unlikely. sIgE to α-gal was detected in all patients (range, 9.7 to >100 kU_A/L). Three patients suffering from adverse reactions to cetuximab nonsuggestive of being allergic served as negative controls for the α-gal-sIgE assay. In addition, all patients showed sIgE to beef, pork, lamb, and rabbit meats but negative sIgE to chicken and turkey meats. We observed a significant correlation between α-gal-sIgE and beef meat-sIgE ($\rho = 0.90$, $P = .03$, Spearman rank test). Furthermore, IgE reactivity to beef was inhibited by α-gal in the 2 patients studied (patients 2 and 3). The responses were dose dependent, but almost complete inhibition was achieved at 100 μg/mL (see Fig E1 in this article's Online Repository at www.jacionline.org). In keeping with previously reported data,[6,7] positive but low levels of sIgE were found against helminths and cat dander in 4 patients. However, negativity of sIgE to nonglycosylated rFel d1 supports the role of α-gal in cat dander IgE reactivity.

The results of these studies definitively show IgE sensitization to α-gal in 5 patients with delayed anaphylaxis to meat. To our knowledge, these are the first reported cases of delayed anaphylaxis due to α-gal allergy in Europe with demonstration of sIgE against this carbohydrate. A history of tick bites, common in rural areas of the studied area (Galicia, Spain), was present in most cases, as previously described.[9] Although the ticks involved were not identified, probably they belonged to the *Ixodes ricinus* species because it is the predominant tick in northwestern Spain[10] where Galicia is located. Furthermore, these results support the diagnostic utility of SPTs with beef extract and/or cetuximab in similar cases. These could be preferable to intradermal tests,[4] which are more sensitive but less specific and have a higher risk of systemic reactions. Further studies are needed to evaluate the prevalence of this type of food allergy in different areas, as well as to further evaluate the proposed diagnostic tests in a larger sample of patients.

Ramón Nuñez, MD[a]
Francisco Carballada, MD, PhD[a]
Arturo Gonzalez-Quintela, MD, PhD[b]
Jose Gomez-Rial, BSc[d]
Manuel Boquete, MD, PhD[a]
Carmen Vidal, MD, PhD[c]

From [a]the Department of Allergy, Hospital Lucus Augusti, Lugo, Spain, and the Departments of [b]Medicine and [c]Allergy and [d]the Laboratory of Immunology, Hospital Clinico Universitario, Santiago de Compostela, Spain. E-mail: carmen.vidal.pan@sergas.es.

The study was supported by the Instituto de Salud Carlos III (Fondo de Investigaciones Sanitarias, Spanish Ministry of Health) grant PI10/01870.

Disclosure of potential conflict of interest: R. Nuñez, F. Carballada, A. Gonzalez-Quintela, J. Gomez-Rial, M. Boquete, and C. Vidal receive research support from Instituto de Salud Carlos III (Spanish Ministry of Health).

REFERENCES

1. Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M, et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-α-1,3-galactose. N Engl J Med 2008;358:1109-17.
2. Marcher BA, Galili U. The Galα1,3Galβ1, 4GlcNAc-R (alpha-gal) epitope: a carbohydrate of unique evolution and clinical relevance. Biochim Biophys Acta 2008; 1780:75-88.
3. Commins SP, Satinover SM, Hosen J, Mozena J, Borish L, Lewis BD, et al. Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in patients with IgE antibodies specific for galactose-α-1,3-galactose. J Allergy Clin Immunol 2009;123:426-33.
4. Jacquenet S, Moneret-Vautrin DA, Bihain BE. Mammalian meat-induced anaphylaxis: clinical relevance of anti-galactose-α-1,3-galactose IgE confirmed by means of skin tests to cetuximab. J Allergy Clin Immunol 2009;124:603-5.
5. Commins SP, Platts-Mills TAE. Anaphylaxis syndromes related to a new mammalian cross-reactive carbohydrate determinant. J Allergy Clin Immunol 2009;124: 652-7.
6. Grönlund H, Adédoyin J, Commins SP, Platts-Mills TAE, van Hage M. The carbohydrate galactose-α-1,3-galactose is a major IgE-binding epitope on cat IgA. J Allergy Clin Immunol 2009;123:1189-91.
7. Arkestäl K, Sibanda E, Thors C, Toyre-Blomberg M, Mduluza T, et al. Impaired allergy diagnostics among parasite-infected patients caused by IgE antibodies to the carbohydrate epitope galactose-α1,3-galactose. J Allergy Clin Immunol 2011;127:1024-8.
8. Commins SP, Platts-Mills TAE. Allergenicity of carbohydrates and their role in anaphylactic events. Curr Allergy Asthma Res 2010;10:29-33.
9. Commins SP, James HR, Kelly LA, Pochan SL, Workman LJ, Perzanowski MS, et al. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose-α-1,3-galactose. J Allergy Clin Immunol 2011; 127:1286-93.
10. Vázquez L, Panadero R, Dacal V, Pato FJ, López C, Diaz P, et al. Tick infestation (Acari: Ixodidae) in roe deer (*Capreolus capreolus*) from northwestern Spain: population dynamics and risk stratification. Exp Appl Acarol 2011;53:399-409.

doi:10.1016/j.jaci.2011.07.020

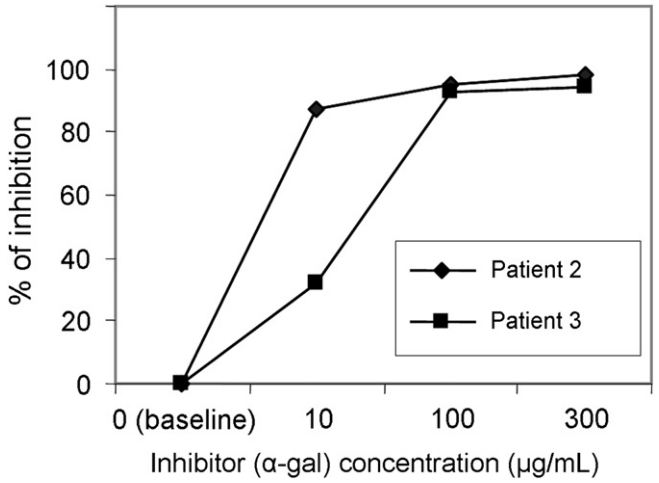

FIG E1. Inhibition assays in 2 patients with delayed meat anaphylaxis and α-gal IgE sensitization (patients 2 and 3). For inhibition assays, 150 μL of serum was preincubated with 150 μL of PBS α-gal solution at 3 dilutions: 10, 100, and 300 μg/mL. After overnight incubation, results were assayed in the UniCAP100 Assay. Results are presented as the percentage of inhibition.